

Functional and histological evaluation of rat sciatic nerve anastomosis using cyanoacrylate and fibrin glue

M.N. Breshah^{a,*}, A.A. Sadakah^a, E.A. Eldrieny^b, K.A. Saad^a

^a Oral & Maxillofacial Surgery Dept., Faculty of Dentistry, Tanta University, Egypt

^b Histology Dept., Faculty of Medicine, Tanta University, Egypt

Abstract

Purpose: The aim of this study is to evaluate both functionally and histologically the validity of the cyanoacrylate and fibrin glue for nerve anastomosing in rat sciatic nerve.

Materials & methods: In this study 45 healthy albino rats were used. In all rats, a unilateral right side sciatic nerve transection was performed and reanastomosed by different methods: Group I (control group): included 15 rats, the anastomosis was done by epineural microsutures using 10/0 nylon. Group II: included 15 rats, the anastomosis was done by using n-butyl-2-cyanoacrylate (Histoacryl®). Group III: included 15 rats, the anastomosis was done by using fibrin glue. Evaluation of nerve recovery was done both functionally and histologically over 3 months postoperatively.

Results: Functional results showed that there was significant difference of the sciatic functional index (SFI) between group I and group II and between group II and group III while there was no significant difference of SFI between group I and group III at the different follow up periods. Histological results showed that after three months there was no complete axonal regeneration in the three groups. The axonal regeneration in group II was less than that in group I and group III. Group II showed foreign body inflammatory reaction and increasing degree of fibrosis. The axonal regeneration was better in group III when compared with group I which showed mild degree of fibrosis.

Conclusion: We can conclude that Fibrin glue was better than the n-butyl-2-cyanoacrylate both functionally and histologically and could be used as an alternative technique to epineural suturing in the microsurgical repair of the transected nerves.

© 2013, Production and Hosting by Elsevier B.V. on behalf of the Faculty of Dentistry, Tanta University.

Open access under [CC BY-NC-ND license](http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords: Peripheral nerve injury; n-Butyl-2-cyanoacrylate; Fibrin glue

1. Introduction

Peripheral nerve injury in the maxillofacial region is a devastating problem with the inferior alveolar, lingual, facial and hypoglossal nerves being the most prone nerves to be affected in this region. These may occur following surgical removal of lower third molar, orthognathic surgery, maxillofacial trauma, dental implant placement, endodontic therapy, facial fractures. Reports suggest that under normal conditions,

* Corresponding author.

E-mail address: maram_nashaat@yahoo.com (M.N. Breshah).

Peer Review under the responsibility of the Faculty of Dentistry, Tanta University

spontaneous nerve fibers healing can be expected within few weeks or months. However, some cases may in fact require surgical intervention [1–3].

Neurotmesis is seen with sharp injury, massive trauma, or severe traction causing nerve rupture. There is loss of nerve trunk continuity with complete disruption of all supporting elements, reinnervation does not occur without surgery [4].

Severe nerve injury has a devastating impact on patient's quality of life. Typical symptoms are sensory and motor function defects that could result in complete paralysis of an affected organ or development of neuropathic pain. Nerve fibers of the transected nerve regenerate spontaneously to the extent limited by the size of the nerve gap, neuroma formation, and scar tissue formation. In the cases of failed recovery, surgical repair of the affected nerve becomes necessary [5].

Different techniques of nerve repair have been used for transected nerve including: neurorrhaphy, gluing, nerve grafts, entubulation [6,7].

Epineural suturing is the most commonly used method for peripheral nerve anastomosis. However, this technique has many disadvantages as excessive handling that leads to increasing trauma and inflammation, difficulty of suturing in confined anatomic locations moreover, suture placement has been thought to cause a hindrance to the sprouting axons and compress the blood supply to the fascicles, thereby impairing the regeneration of transected nerve ends after repair. These factors lead to the development of various tissue adhesives for the purpose of atraumatic tissue repair [8–10].

Using of tissue adhesives (fibrin glue and cyanoacrylate glue) for microneural anastomosis seems attractive based on the theoretical advantage of less tissue handling and consequent less trauma, and more excellent coaptation of the severed nerve fascicles. Moreover, a sutureless repair is possible, even when access is poor in confined anatomic locations [11,12].

The synthetic cyanoacrylate adhesives are liquid monomers that on contact with various substances, such as water or blood, polymerize into long chains, creating a flexible film that holds the apposed wound edges together [13,14].

Cyanoacrylate adhesives are inexpensive, relatively easy to apply, do not carry any risk of viral transmission and have sufficient strength to maintain a nerve anastomosis, even under tension, less tissue handling and consequent trauma, useful in confined anatomical locations [12,15].

Fibrin glue is a plasma-derived biologic concentrate of topical use, its mechanism of action is similar to the last stage of the physiological coagulation (fibrin

formation) when a solution of plasma fibrinogen is activated by thrombin resulting in the formation of a semirigid to rigid fibrin clot that consolidates and adheres to the application site and hold tissues and materials in a desired configuration [16].

In contrast to synthetic cyanoacrylate adhesive, fibrin glue has the significant advantage of being biocompatible and biodegradable, without inducing inflammation, foreign body reactions, tissue necrosis or extensive fibrosis, promote angiogenesis and local tissue growth and repair [17,18].

Palazzi et al., [11] and Suri et al., [8] demonstrated that fibrin glue is a sealant and not a nerve barrier and there is no appreciable clot retraction because the sealant does not contain thrombocytes.

Because of the contradictory opinions regarded nerve repair with limited comparative data, accordingly this study was conducted to evaluate nerve anastomosis by using cyanoacrylate and fibrin glue both functionally and histologically.

2. Materials & methods

Fourty five healthy albino rats weighting 280 to 300 g were used in this study. All rats underwent a preoperative clinical evaluation by using a simplified version of SFI to evaluate functional recovery, animals was allowed to become conditioned to the walking track. Their hind foot was dipped in an ink solution and they were permitted to walk down the track upon a strip of white paper. The prints by the ink were left to dry and then analyzed as described by Ref. [19]. The measurements of the foot prints were:

- *Toe spread*: The distance from the first to the fifth toe (TS).
- *Intermediate toe spread*: The distance from the second to the fourth toe (ITS).

2.1. Surgical procedures

The rats were operated under general anesthesia with intramuscular injection of 50 mg/kg of body weight ketamine.¹

According to Suri et al., [8] and Martins et al., [20] hair was shaved from the hind limb and mid back, the rats were placed in prone position. The sciatic nerve was exposed at the dorsocaudal region. An incision was made starting 0.5 cm laterally from the animal's midline and extending laterally for 3 cm toward the tibiofemoral

¹ Ketam. Egyptian Int. pharm. IND. Co. EIPICO.



Fig. 1. Repaired sciatic nerve with n-butyl-2-cyanoacrylate.

articulation. The femoral biceps and gluteus muscles were separated using blunt dissection to allow access to provide exposure of the sciatic nerve. Unilateral right side sciatic nerve transection was performed by using no.11 blade. In group I (Control Group) The nerve was repaired by epineural microsutures using 10/0 nylon² under magnification of binocular loupe.

In group II the nerve was repaired using n-butyl-2-cyanoacrylate (Histoacryl®).³ Nerve edges were apposed and hold in apposition while applying the adhesive and for approximately 30 s after application to allow it to cure and to prevent seepage between edges (Fig. 1).

In group III the nerve was repaired using fibrin glue.⁴ The two main components of the fibrin glue were applied to the nerve edges by a double syringe applicator to permit that the fibrinogen and the thrombin concentrates are mixed passively at the end of the delivery needle just prior to contact with the repair site (Fig. 2).

In all rats, the wound was closed in layers and the skin was painted with povidone iodine antiseptic solution. The rats were kept in their cages with free access to water and food until complete recovery.

(1) Functional evaluation:

All rats underwent a postoperative sciatic functional index (SFI) records at the following intervals after 1,2,3 weeks and 1,2,3 months.



Fig. 2. Repaired sciatic nerve with fibrin glue.

(2) Histological evaluation:

Rats from each group were sacrificed with an over dose of intramuscular injection of ketamine after 1,2,3 months. A fifteen mm segment was excised of the sciatic nerve including the repaired portion and the proximal as well as distal stumps which were prepared for histological study under light and transmission electron microscope.

2.2. Preparation of semi-thin and ultra-thin sections

According to Dawes [21] and Hayat [22], specimens were immediately fixed in 2.5% phosphate buffered glutaraldehyde for 2 h then post fixed in freshly prepared 1% phosphate buffer osmium tetroxide for 1 h after dehydration the specimen infiltrated with the epoxy resin mixture then embedded in capsules filled with fresh epoxy resin mixture and polymerized in an oven for one day at 65 °C. For semithin sections, 0.5–1 µm thick sections were cut and were mounted on glass slides then stained with toluidine blue. These semithin sections were examined with light microscope. For ultrathin sections, sections of 80–100 nm thick were cut. These sections were either of grey or silver interference color. They were picked upon 200 mesh naked copper grids. The mounted sections were double stained, first with uranyl acetate solution for 30 min, then stained with lead citrate for 5–10 min, washed with distilled water and dried upon clean filter paper. Finally, each grid was examined and photographed using JEOL-JEM 100 SX electron

² FSSB Chirurgische Nadein GmbH, Allmendweg 2, 79798, Jestetten, Germany.

³ B. Braun, Am Aesculap- platz, 78532, Tuttlingen, Germany.

⁴ Al-Shabrawishi Hospital Blood Bank, Cairo Egypt.

microscope, Japan at 80 kilo vol at EM unit in Tanta University.

3. Results

Only 31 rats survived the entire study period. They all tolerated the surgical procedure planned for each group without complications as well as the follow up period until the intended date of sacrifice. 7 rats died within the first week without any obvious cause, the other 7 rats underwent an autotomy of their fingers and were excluded from the study. Immediately after the transection of the sciatic nerve, a complete paralysis of the operated right side foot was observed in all rats. Through the follow up of all rats, no wound dehiscence or infection were noticed in any of the rats of all groups. All rats showed some weakness in their operated feet which decreased by time.

3.1. Gross appearance of the repaired sciatic nerve

All the sciatic nerves of groups I, II and III showed complete healing of the transected site. Dissection and exposure of the sciatic nerves at the dates of sacrifice (at 1,2,3 months) showed adhesions with the surrounding tissues. Nylon suture material and n-butyl-2-cyanoacrylate could be detected in the repaired area at the dates of sacrifice while fibrin glue was completely resorbed. Signs of inflammation could be detected in one rat in group II on three months post-operative.

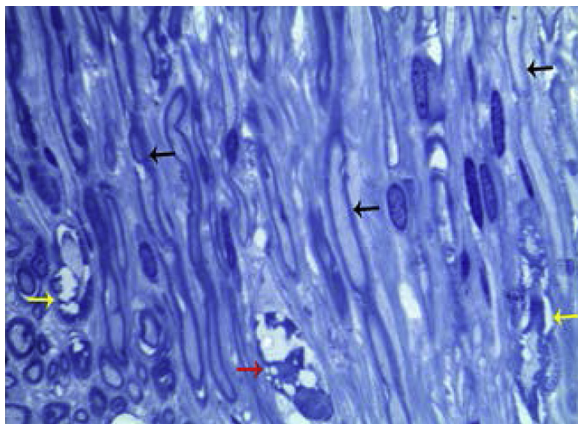


Fig. 3. Photomicrograph of toluidine blue stained longitudinal section showing sciatic nerve of (group I), 3 months post-operative showing regeneration of most fibers and uniform arrangement of them (black arrows) and reduction of spaces between them, still few areas of degenerated fibers (red arrow) and others with myelin ballooning (yellow arrows) (magnification $\times 1000$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Histological examination

The axonal degeneration was evident in the first month while, the morphological picture seen on the second month was more organized with decreased areas of degenerated fibers. By the end of the study period, after three months there was no complete axonal regeneration in the three groups. The axonal regeneration was more better in group III when compared with group I and also, group I showed more deposition of collagen fibers which was indicative of more fibrosis (Fig. 6). The axonal regeneration in group II was less than that in group I and group III (Figs. 3–5) in addition to the presence of foreign body inflammatory reaction which represented by the infiltration of mast cells and eosinophils and increasing degree of fibrosis that represented by the deposition of more collagen fibers in group II Figs. 7 and 8.

3.2.1. Statistical analysis

The paired *t*-test was performed to analyze the change by time in each group and ANOVA and TUKEY'S tests were used for the comparison between mean T.S and I.T.S in groups I, II and III.

3.2.2. Regarding T.S in the three groups preoperatively and postoperatively (after 3 months)

In group I (control group) Differences between preoperative and postoperative (after 3 months) mean \pm SD was 1.82 ± 0.25 . In group II Differences

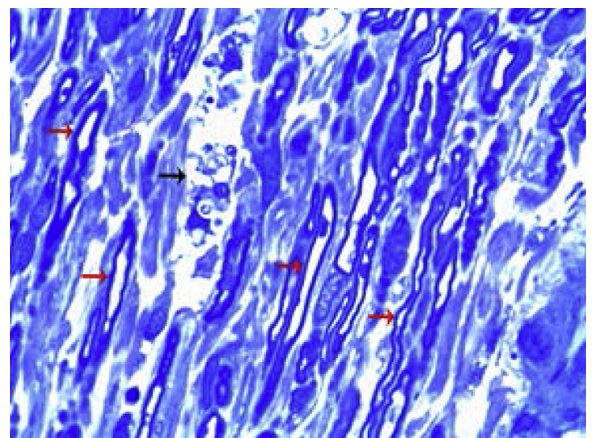


Fig. 4. Photomicrograph of toluidine blue stained longitudinal section showing sciatic nerve of (group II), 3 months post-operative showing more regenerating fibers (red arrows) and still degenerating fibers (black arrows) with increasing degree of fibrosis between fibers (magnification $\times 1000$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

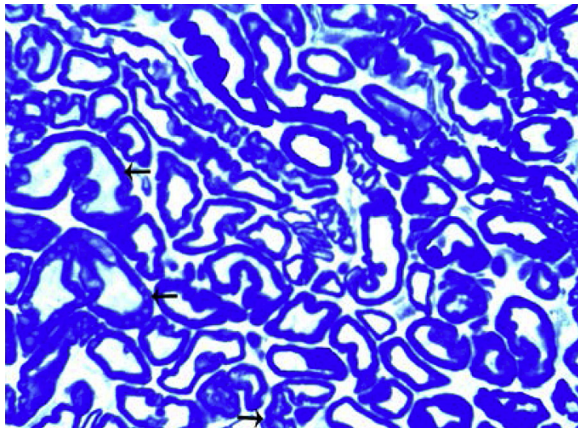


Fig. 5. Photomicrograph of toluidine blue stained oblique section showing sciatic nerve of (group III), 3 months postoperative showing regeneration of most fibers and few fibers with focal lysis of myelin (black arrows) (magnification $\times 1000$).

between preoperative and postoperative (after 3 months) mean \pm SD was 3.45 ± 0.44 . In group III Differences between preoperative and postoperative (after 3 months) mean \pm SD was 1.05 ± 0.28 , (Table 1 & Fig. 9).

3.2.3. Concerning of I.T.S in the three groups preoperatively and postoperatively (after 3 months)

In group I (control group) Differences between preoperative and postoperative (after 3 months) mean \pm SD was 1.77 ± 0.26 . In group II Differences between preoperative and postoperative (after 3 months) mean \pm SD was 3.45 ± 0.44 . In group III Differences between preoperative and postoperative

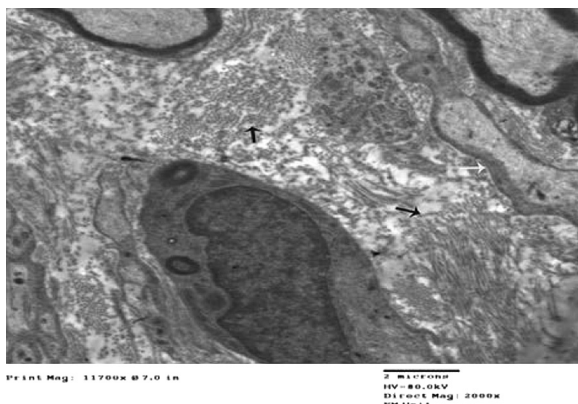


Fig. 6. Electron micrograph of longitudinal section in a rat sciatic nerve, (group I), 3 months post-operative showing nerve fiber during myelination (white arrow) and more increasing in the degree of fibrosis (black arrows) (TEM, magnification $\times 2000$).



Fig. 7. Electron micrograph of longitudinal section in a rat sciatic nerve, (group II), 2 months post-operative showing infiltrating eosinophills (red arrow) with its extracellular granules (black arrow) (TEM, magnification $\times 1000$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(after 3 months) mean \pm SD was 1.25 ± 0.68 , (Table 2 & Fig. 10).

4. Discussion

Nerve injury overerupted many problems as in neurotmesis which is seen with sharp injury, massive trauma, or severe traction causing nerve rupture. There is loss of nerve trunk continuity with complete disruption of all supporting elements so, reinnervation does not occur without surgery [4,5].



Fig. 8. Electron micrograph of a section in a rat sciatic nerve, (group II), 2 months post-operative showing an infiltrating mast cell (red arrow) with its extracellular granules (white arrow) and heavy fibrosed area (black arrow) (TEM, magnification $\times 1500$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Preoperative and postoperative (after 3 months) statistical analysis of T.S in the three groups.

TS	Preoperative	After 3 mths	Paired <i>t</i> -test	
	Mean \pm SD	Mean \pm SD	<i>t</i>	<i>P</i> -value
Group I	17.09 \pm 0.83	15.27 \pm 0.85	23.90	0.00
Group II	17.00 \pm 0.82	13.55 \pm 0.64	24.92	0.00
Group III	17.00 \pm 0.82	15.95 \pm 0.72	11.70	0.00

Anastomosis with epineural suturing is the reference standard for the repair of an injured nerve while the use of tissue adhesives for microneural anastomosis seems attractive to avoid the disadvantages of epineural suturing [23,8].

Recovery of injured sciatic nerve is measured using morphological, electrophysiological and functional tests. Although the morphological and electrophysiological studies provide valuable measures, it didn't necessarily correlate with the return of function so, functional recovery is the primary goal to be studied to evaluate nerve regeneration (Dellon and Mackinnon [24] and Munro et al. [25]) also, the application of histomorphometric analysis could yield false positive results (Swallow et al. [26] and Varejao et al. [27]), accordingly, we have evaluated recovery through functional together with histological evaluation through light and electron microscope.

Sarikcioglu et al. [28] reported that sciatic functional index (SFI) provides a non-invasive method of assessing the functional status of the sciatic nerve during the regeneration process because proper walking requires coordinated function involving sensory input, motor response and cortical integration.

Since its introduction by De Medinaceli et al. [29], the sciatic functional index has become the foundation to assess the overall functional recovery after sciatic nerve injury. This method was later modified by Carlton and Goldberg [30] and Bain et al. [31]. However, walking track reliability on quantification of SFI

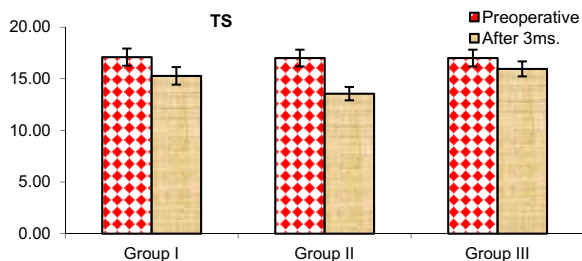


Fig. 9. Bar graph representing T.S preoperatively and postoperatively (after 3 months) in the three groups.

Table 2

Preoperative and postoperative (after 3 months) statistical analysis of I.T.S in the three groups.

ITS	Preoperative	After 3 mths	Paired <i>t</i> -test	
	Mean \pm SD	Mean \pm SD	<i>t</i>	<i>P</i> -value
Group I	12.27 \pm 1.56	10.50 \pm 1.47	22.52	0.00
Group II	11.80 \pm 1.40	8.35 \pm 1.18	24.92	0.00
Group III	11.90 \pm 1.37	10.65 \pm 1.03	5.84	0.00

after severe nerve injury can be compromised by the development of flexion contracture, dragging of the tail, or variability in print length with gait velocity. Additionally, the value of SFI according to the equation developed by De Medinaceli, ranges from zero (normal rats) to -100 (total impairment), the fact that the SFI was never zero in healthy, intact animals probably indicates that although reliable, the method is not entirely precise; this could be due to either incorrectness of the formula used or to a potential error in its application (Dijkstra et al. [32] and Varejao et al. [27]), accordingly, we have used a simplified version of the sciatic functional index to evaluate the functional recovery as this gives characteristic foot measurements about the associated neuromuscular dysfunction as described by Haapaniemi et al. [19].

Rats subjected to an autotomy of their fingers were excluded from our study as these rats will not give an accurate foot prints or accurate sciatic functional index, this was in agreement with the studies achieved by Kauppila [33].

The current results revealed that there was a significant decrease in the values of toe spread (T.S) and intermediate toe spread (I.T.S) postoperatively in group I and then significantly increased gradually but not reach to the preoperative values and this coincide with Haapaniemi et al. [19] who reported that the distance decreased down to around 35% and 53% of the preoperative values for the T.S. and I.T.S. respectively of nerve repair with epineural suture with no recovery to the preoperative values.

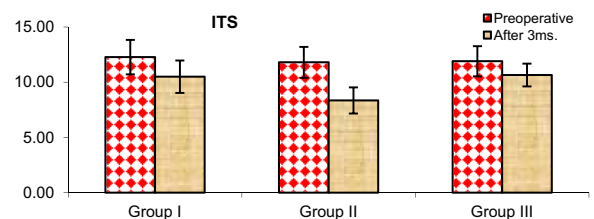


Fig. 10. Bar graph representing I.T.S preoperatively and postoperatively (after 3 months) in the three groups.

Up to our knowledge sciatic functional index had not been used in any study to evaluate the efficacy of fibrin glue or n-butyl-2-cyanoacrylate for nerve repair.

These results showed that there was a significant difference of the values of T.S and I.T.S between group I and group II and between group II and group III at the different follow up periods, while there was no significant difference of these values between group I and group III at the different follow up periods.

In our study we did not find any case of anastomotic failure in group I and group III at the different follow up periods and this is in agreement with the findings of Attar et al. [34] who reported that suture and fibrin glue were equally adequate to stabilize the reanastomosed nerves during regeneration. Similarly, there was a significant nerve repair and anastomosis at the different follow up periods in group II, this is in agreement with the results achieved by Choi et al. [12] and Elgazzar et al. [35].

The morphological findings showed that the axonal degeneration was evident in the first month while, the morphological picture seen on the second month was more organized with decreased areas of degenerated fibers. By the end of the study period, after three months there was no complete axonal regeneration in the three groups, this is according to the studies of Elgazzar et al. [35] on suture and cyanoacrylate reanastomosis and Sandrini et al. [10] on fibrin glue reanastomosis, both found that three months after reanastomosis, showed histologically less nerve regeneration indicating a partial regeneration of the nerve fibers.

Also, it was observed that the axonal regeneration was more better in group III when compared with group I and also, group I showed more deposition of collagen fibers which was indicative of more fibrosis and this was in agreement of the findings achieved by Feldman et al. [36] who reported that the amount of axonal regeneration and alignment were superior with the fibrin adhesive technique which also showed less fibrosis compared with epineural suturing.

Also, it was found that the axonal regeneration in group II was less than that in group I and group III in addition to the presence of foreign body inflammatory reaction which represented by the infiltration of mast cells and eosinophills and increasing degree of fibrosis that represented by the deposition of more collagen fibers in group II when compared with group I and group III. This was in agreement with Weiken et al. [37] who found that the cyanoacrylate causes a foreign body inflammatory reaction and retractile fibrosis.

There was no correlation between the morphological findings and the functional outcomes between group I and group III, this is comparable to the findings of Algora et al. [38] and Sarikcioglu et al. [28] who found that a parallel relationship does not always exist between functional recovery and axonal regeneration. It is possible to have good axonal regeneration but poor function therefore, morphological evaluation is not thought to be the most reliable way to evaluate the outcome of nerve repair because of mismatching, separation, protruding or kinking between proximal and distal axons.

From the results of this study it was concluded that the application of both n-butyl-2-cyanoacrylate and fibrin glues was easier, simpler and less time consuming, fibrin glue was superior to the n-butyl-2-cyanoacrylate both functionally and histologically and could be used as an alternative technique to epineural suturing in the microsurgical repair of the transected nerves in addition to that the functional recovery is the primary goal to be studied to evaluate nerve regeneration although the morphological study through histological evaluation provide valuable measures, it didn't necessarily correlate with the return of function.

References

- [1] Robinson PP, Loescher AR, Smith KG. A prospective, quantitative study on the clinical outcome of lingual nerve repair. *Br J Oral Maxillofac Surg* 2000;38:255–63.
- [2] Queral-Godoy E, Valmaseda-Castellon E, Berrini-Aytes L, Gay-Escoda C. Incidence and evaluation of inferior alveolar nerve lesions following lower third molar extraction. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004;6:1–7.
- [3] Libersa P, Savignat M, Tonnel A. Neurosensory disturbances of the inferior alveolar nerve: a retrospective study of complaints in a 10-year period. *J Oral Maxillofac Surg* 2007;65:1486–9.
- [4] Campbell WW. Evaluation and management of peripheral nerve injury. *Clin Neurophysiol* 2008;119:1951–65.
- [5] Matsuyama T, Mackay M, Midha R. Peripheral nerve repair and grafting techniques: a review. *Neurol Med Chir (Tokyo)* 2000;40:187–99.
- [6] Miloro M. *Microneurosurgery*. Peterson's principles of oral and maxillofacial surgery. 2nd ed. BC Decker; 2004. p. 819–37.
- [7] Bhandari PS, Sadhotra LP, Bhargava P, Bath AS, Mukherjee MK, Bavdekar RD. What is new in peripheral nerve repair? *Ind J Neurotrauma* 2007;4(1):21–3.
- [8] Suri A, Mehta VS, Sarkar C. Microneural anastomosis with fibrin glue: an experimental study. *Neurol India* 2002;50:23–6.
- [9] Wolford LM, Stevao EL. Considerations in nerve repair. *BUMC Proc* 2003;16:152–6.
- [10] Sandrini F, Jurrior E, Escoda C. Rabbit facial nerve anastomosis with fibrin glue: nerve conduction velocity evaluation. *Bras J Otorrinolaringol* 2007;73(2):196–201.
- [11] Palazzi S, Vila-Torres J, Lorenzo JC. Fibrin glue is a sealant and not a nerve barrier. *J Reconstr Microsurg* 1995;11:135–9.

- [12] Choi BH, Kim BY, Huh JY, Lee SH, Zhu SJ, Hug JH, et al. Microneuronal anastomosis using cyanoacrylate adhesives. *Int J Oral Maxillofac Surg* 2004;33:777–80.
- [13] Esposito C, Damiano R, Settimi A, De Marco M, Maglio P, Centonze A. Experience with the use of tissue adhesives in pediatric endoscopic surgery. *Surg Endosc* 2004;18:290–2.
- [14] Petersen B, Barkun A, Carpenter S, Chotiprasidhi P, Chuttani R, Silverman W, et al. Tissue adhesives and fibrin glues. *Gastrointest Endosc* 2004;60:327–33.
- [15] Landegren T, Risling M, Brage A, Persson JK. Long-term results of peripheral nerve repair: a comparison of nerve anastomosis with ethyl-cyanoacrylate and epineural sutures. *Scand J Plast Reconstr Surg Hand Surg* 2006;40:65–72.
- [16] Burnouf T, Radosevich M, Goubran H. Local hemostatic blood products: fibrin sealant and platelet gel. *World Federation of Hemophilia (WFH)*; 2004. No. 36.
- [17] Dvorak HF, Harvey VS, Estrella P, Brown LF, McDonagh J, Dvorak AM. Fibrin containing gels induce angiogenesis. Implications for tumor stroma generation and wound healing. *Vox Sang* 1987;57:673–86.
- [18] Kram HB, Nathan RC, Mackabee JR, Klein SR, Shoemaker WC. Clinical use of nonautologous fibrin glue. *Am Surg* 1988;54:570–3.
- [19] Haapaniemi T, Nishiura Y, Dahlin LB. Functional evaluation after rat sciatic nerve injury followed by hyperbaric oxygen treatment. *J Peripher Nerv Syst* 2002;7:149–54.
- [20] Martins RS, Siqueira MG, Da Silva CF, Plese JPP. Overall assessment of regeneration in peripheral nerve lesion repair using fibrin glue, suture, or a combination of the 2 techniques in a rat model. Which is the ideal choice? *Surg Neurol* 2005;64:S1:10–S1:16.
- [21] Dawes CJ. Biological techniques for transmission electron microscopy. 1st ed. Ladd. Res. Industries, Inc. Pub.; 1980. p. 16.
- [22] Hayat MA. Basic techniques for transmission electron microscopy. New York: Harcourt Brance Jovanich. Co; 1987. p. 332.
- [23] Millesi H. Progress in peripheral nerve reconstruction. *World J Surg* 1990;14:733–47.
- [24] Dellon AL, Mackinnon SE. Selection of the appropriate parameter to measure neural regeneration. *Ann Plast Surg* 1989;23:197–202.
- [25] Munro CA, Szalai JP, Mackinnon SE, Medha R. Lack of association between outcome measures of nerve regeneration. *Muscle Nerve* 1998;21:1095–7.
- [26] Swallow RK, Leiser MR, Fink BE, Sauer C, Nicklous DM, Hoffman JR. Anatomical but not functional recovery from a sciatic nerve crush is enhanced by treatment with testosterone. *Restorative Neurol Neurosci* 1999;15:297–303.
- [27] Varejao ASP, Cabrita AM, Meek MF, Bulas-Cruz J, Melo-Pinto P, Raimondo S, et al. Functional and morphological assessment of a standardized rat sciatic nerve crush injury with a non-serrated clamp. *J Neurotrauma* 2004;21:1652–70.
- [28] Sarikcioglu L, Demirel BM, Utuk A. Walking track analysis: an assessment method for functional recovery after sciatic nerve injury in the rat. *Folia Morphol* 2009;68(1):1–7.
- [29] De Medinaceli L, Freed WJ, Wyatt RJ. An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. *Exp Neurol* 1982;77:185–92.
- [30] Carlton JM, Goldberg NH. Quantitating integrated muscle function following reinnervation. *Surg Forum* 1986;37:611–2.
- [31] Bain JR, Mackinnon SE, Hunter DA. Functional evaluation of complete sciatic, peroneal and posterior tibial nerve lesions in the rat. *Plast Reconstr Surg* 1989;83:129–36.
- [32] Dijkstra JR, Meek MF, Robinson PH, Gramsbergen A. Methods to evaluate functional nerve recovery in adult rats: walking track analysis, video analysis and the withdrawal reflex. *J Neurosci Methods* 2000;96:89–96.
- [33] Kaupila T. Correlation between autotomy-behavior and current theories of neuropathic pain. *Neurosci Biobehav Rev* 1998;23:111–29.
- [34] Attar BM, Zalzal H, Razavi M, Ghoreishian M, Rezaei M. Effectiveness of fibrin adhesive in facial nerve anastomosis in dogs compared with standard microsuturing technique. *J Oral Maxillofac Surg* 2012;70:2427–32.
- [35] Elgazzar RF, Abdulmajeed I, Mutabbakani M. Cyanoacrylate glue versus suture in peripheral nerve reanastomosis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;104:465–72.
- [36] Feldman MD, Staloff RT, Epteinn G, Ballas SK. Autologous fibrin tissue adhesive for peripheral nerve anastomosis. *Arch Otolaryngol Head Neck Surg* 1987;113:963–7.
- [37] Wicken K, Angioi-Duprez K, Lim A, Marchel L, Merel M. Nerve anastomosis with glue: comparative histologic study of fibrin and cyanoacrylate glue. *J Reconstr Microsurg* 2003;19(1):17–20.
- [38] Algora J, En Chen L, Seaber AV, Wong GH, Urbaniak JR. Functional effects of lymphotoxin on crushed peripheral nerve. *Microsurgery* 1996;17:131–5.